Figure 2

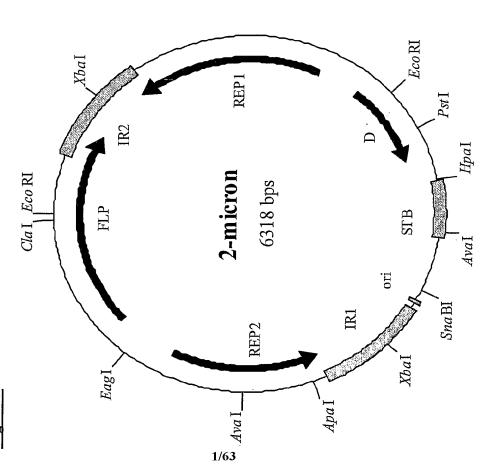
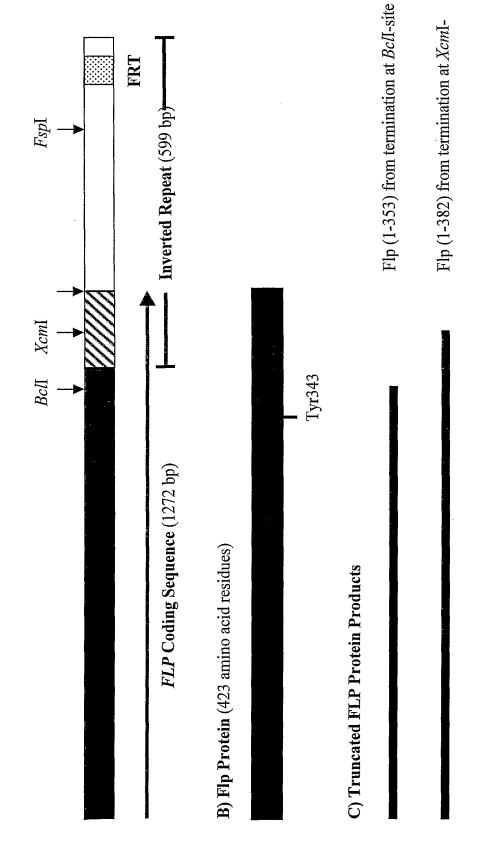


Figure 1

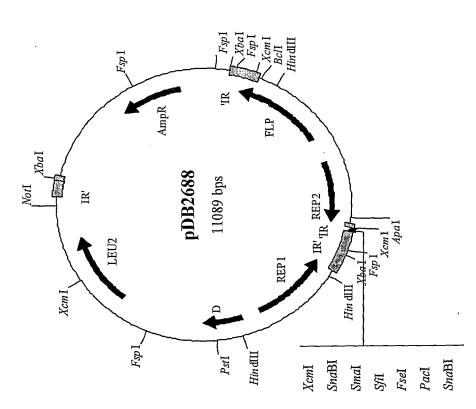
Figure 3

A) Restriction Endonuclease Sites used for DNA Insertions in FLP and the FLP Inverted



Hindll | Kant | Ball |

Figure 5



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Figure 7

Figure 6

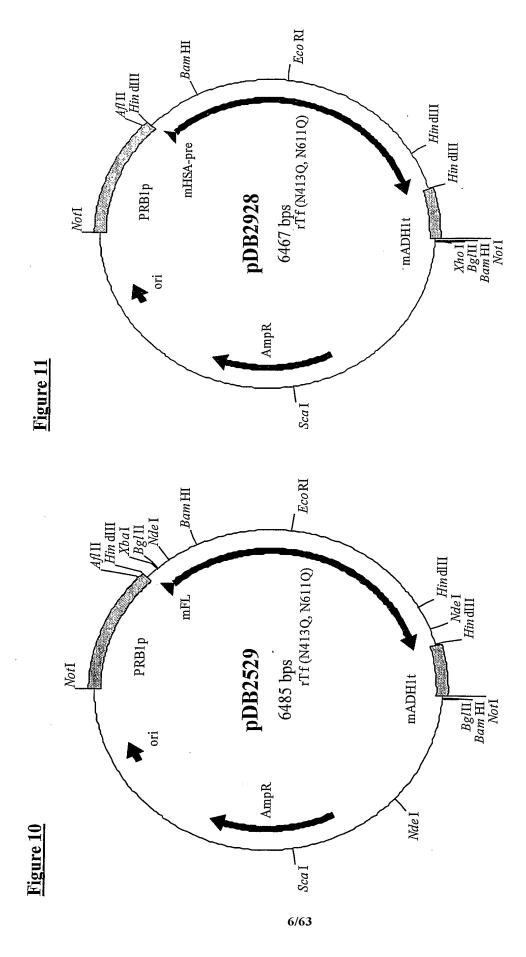
mFL = modified HSA(pre)/MF α 1(pro) fusion leader sequence Xbal IR Xbal ||BglII |NdeI |Bam HI mADHIt AmpR ' | Nde I | BcII | Hin dIII Modified Tf 띮 PRB1p , mFL SphI FLP 16292 bps pDB2711 NotI REP2 S_{FSEI} F_{RSEI} P_{RSEI} A_{PREI} REP1 $Hin \, dIII^{'}/BclI_{I}$ ĸ $egin{array}{c} BcII', \ StuI \ BgIII \end{array}$ Pst1, Stu1, Hin dIII. Xbal Hin dIII Stu I

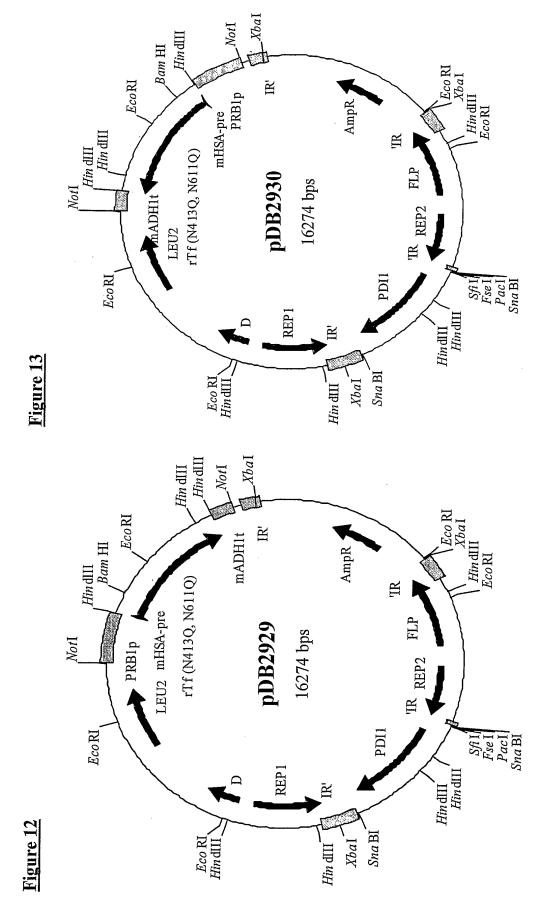
Xcm I BcII Hin dIII K FLP AmpR Apal Xcm I Sna BI PacI Fse I Sfi I REP2 XbaI 'IR $|HindII|^2$ BcIIpDB2690 13018 bps NotI PDI1 HindIII LEU2 R Xcm I REP1 XbaI FspI Acm I SnaBI BcIIHin dIII FspI PstI Hin dIII_

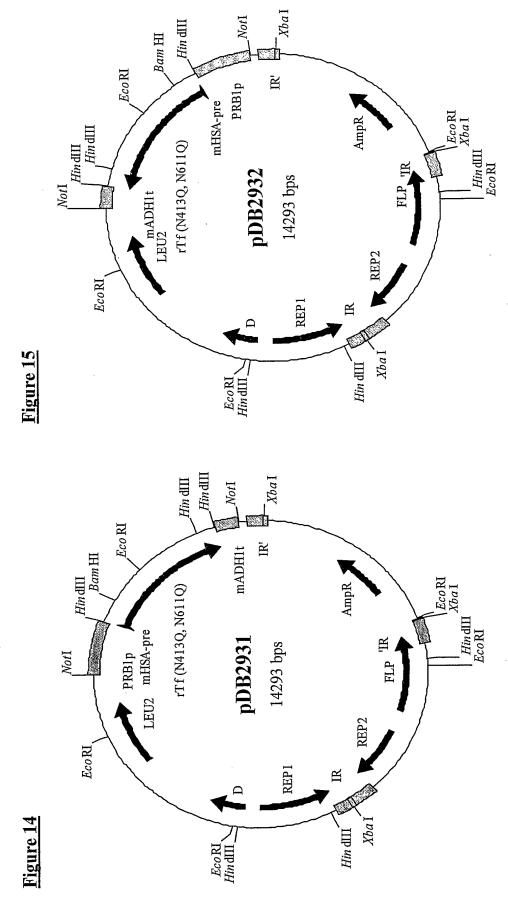
Figure 8

Figure 9

Bam HI Eco RI Ndel 4ft II Hin dIII AccI mHSA-pre = modified HSA-pre leader sequence Stul rTf (N413Q, N611Q) mHSA-pre HindIII PRB1p NdeIAcc I Hin dIII Bam HI pDB2921 5858 bps AmpR Scal mFL = modified HSA(pre)/MF α 1(pro) fusion leader sequence Bam HI EcoRI III dIII Xba I Bg[II Modified Tf mFLHindIII PRB1p pDB2515 5876 bps Bam HI Hin dIII AmpR Xmn I Scal







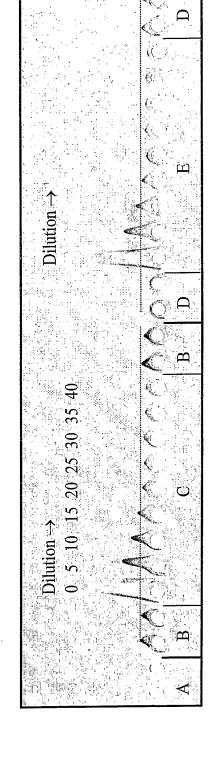
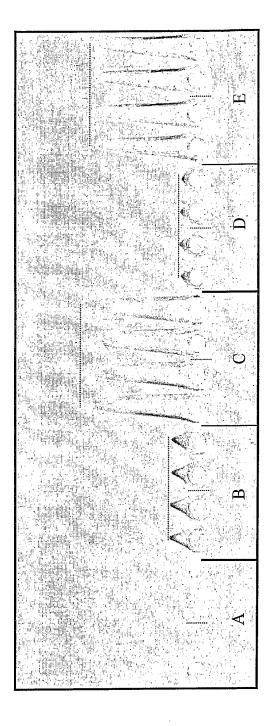


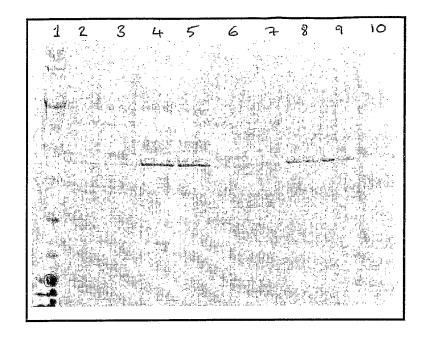
Figure 16



igure 17

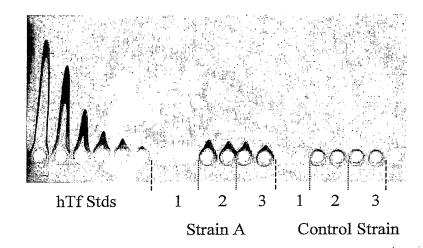
Figure 18

5



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Figure 19



5

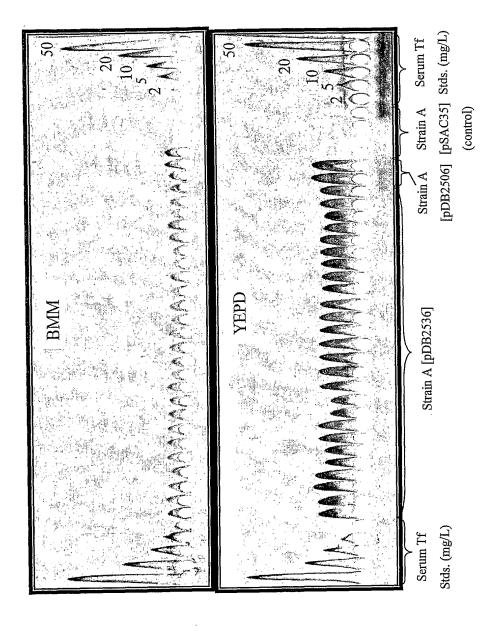
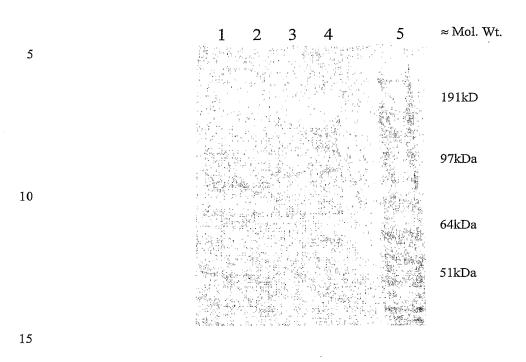


Figure 21



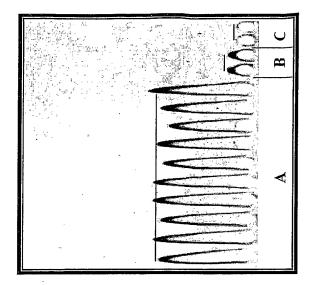
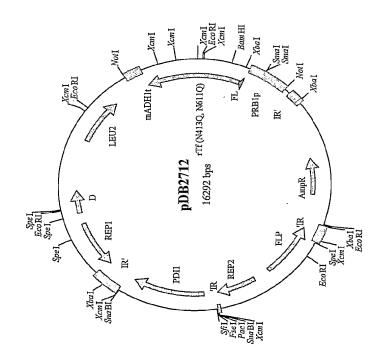
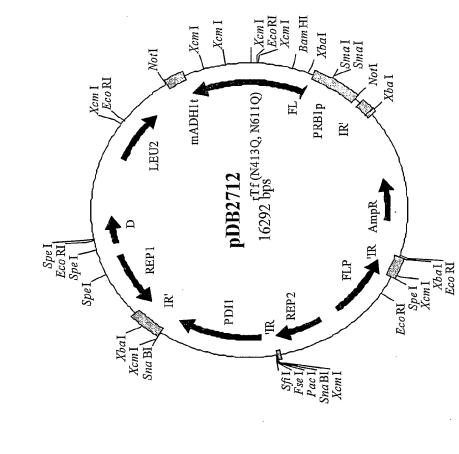


Figure 2.





≈ Mol.
Wt.

1 2 3 4 5 6
Wt.

97kDa

97kDa

51kDa

39kDa

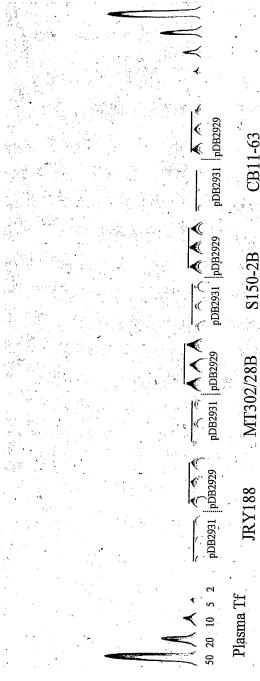
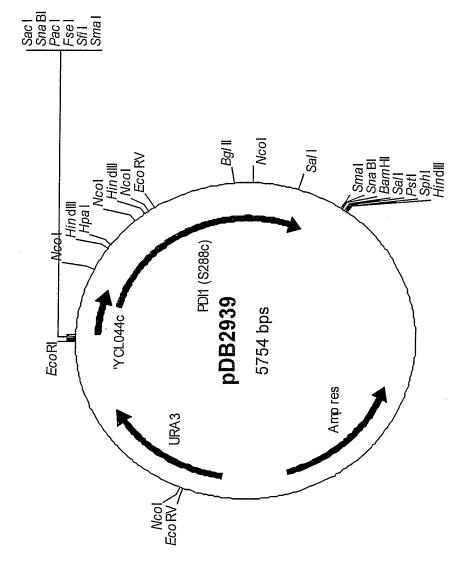
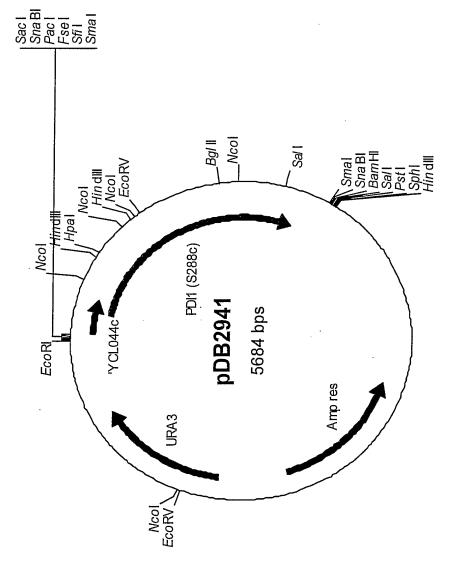


Figure 2



ligure 27



igure 28

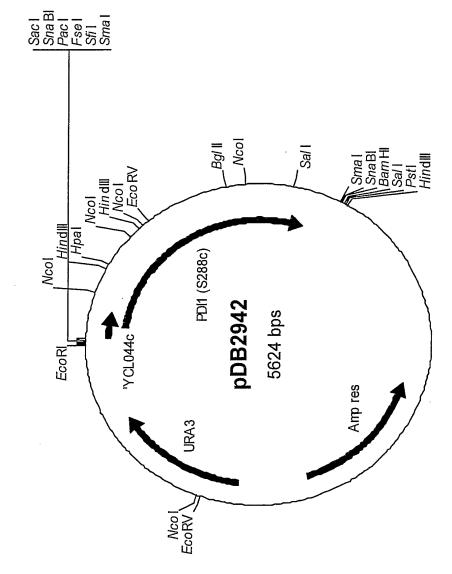


Figure 29

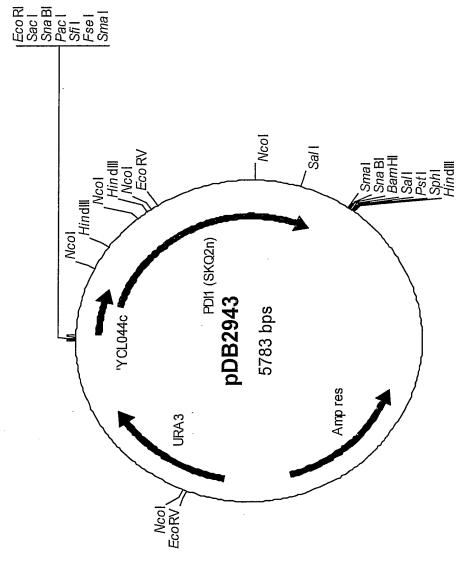


Figure 30

Eco RV Nool Hindill Small Sphi Small Hindill Small Hindill Small Hindill Small Hindill Small Hindill Small Hindill Small RCL044c

Small Small Small Small Small Eco RV Nool Small Eco RV Nool

EcoRV
Ncol
Hindlil
Ncol
Ncol
Hindlil
Saul
Saul
Saul
FDI1 (SKQ2n)

PDB2963

Hindlil
Small
Fsel
Small
Fsel
Small
Fsel
Small
Fsel

Figure 31

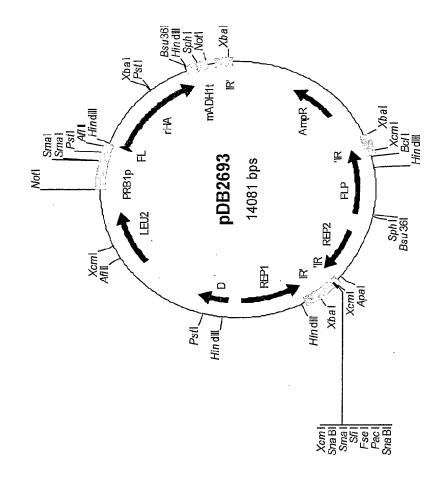
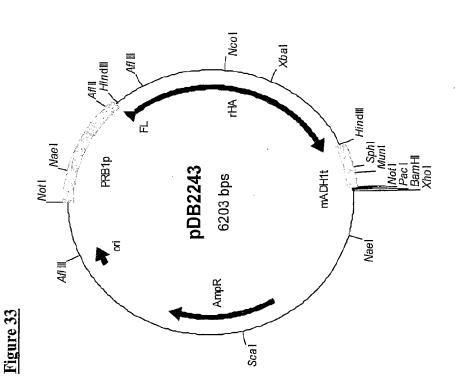
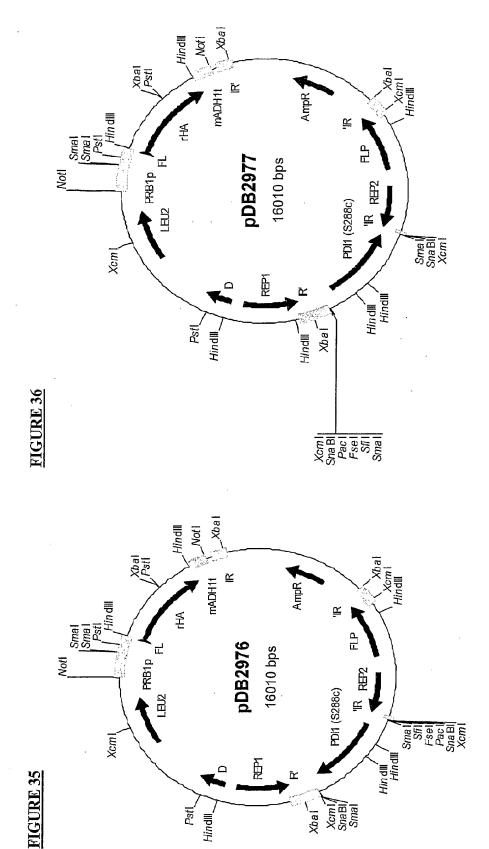
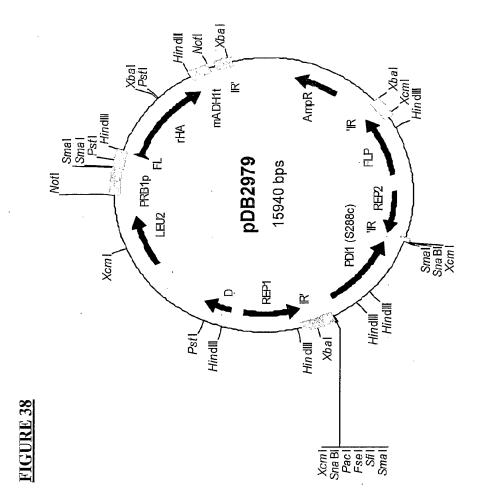


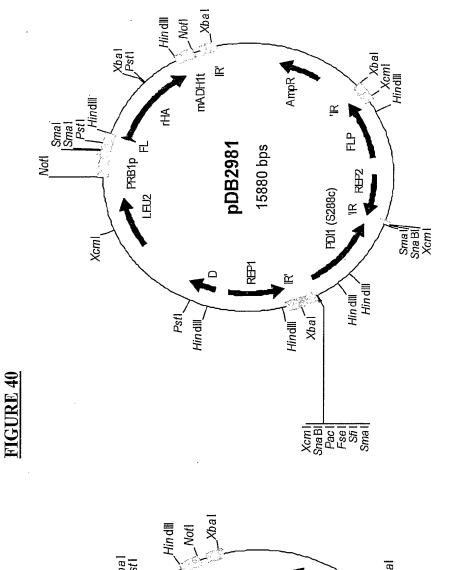
Figure 34



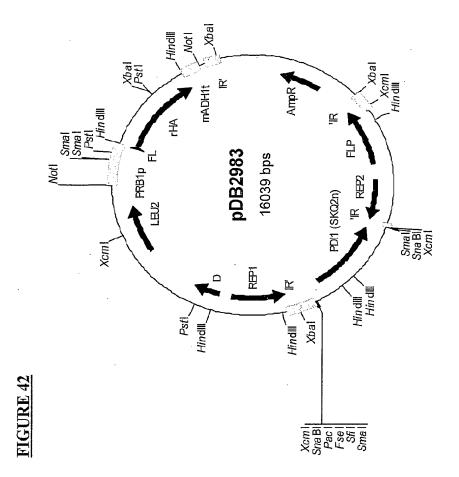


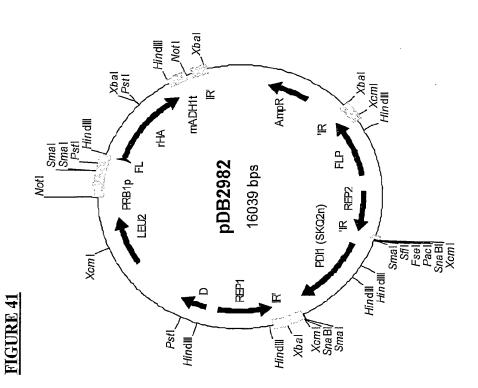


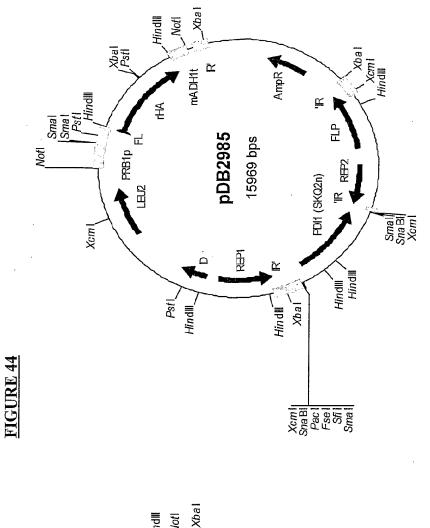
Pest | Smal | FLD | Facil | Fa

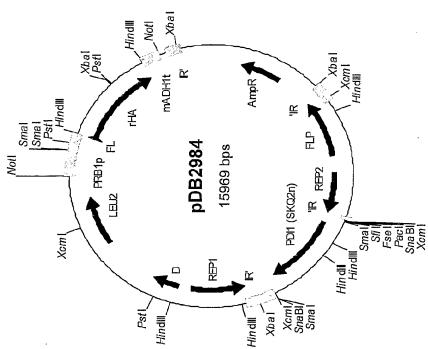


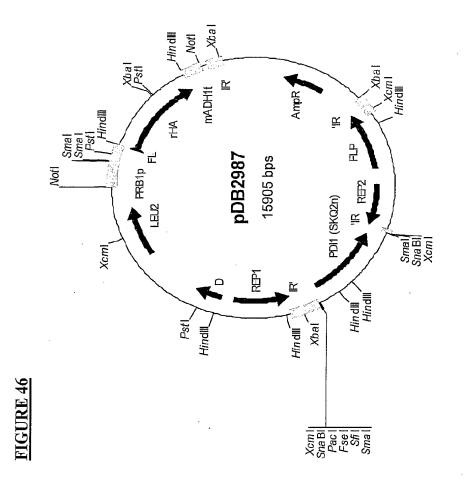
Amale Small Small

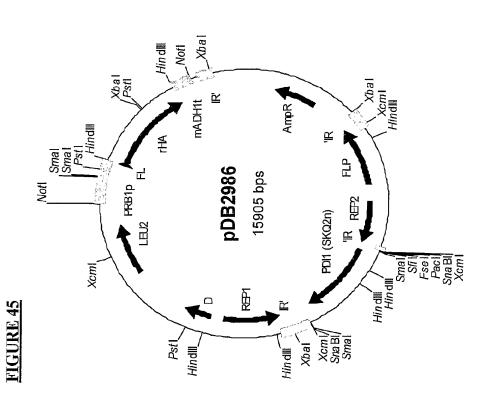


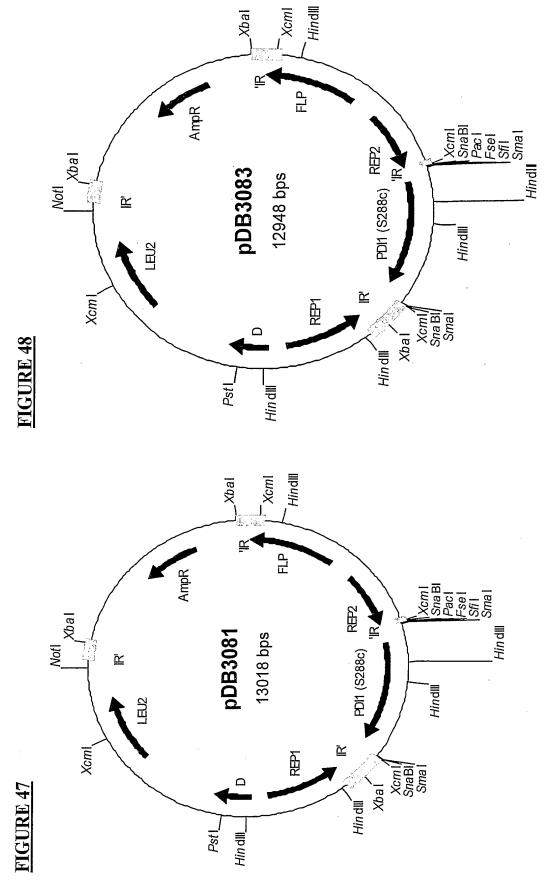


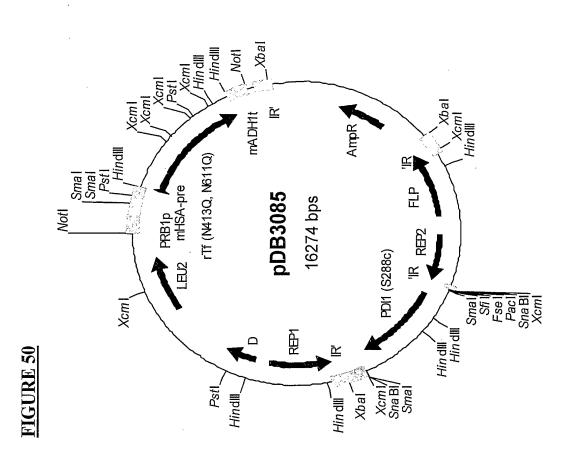


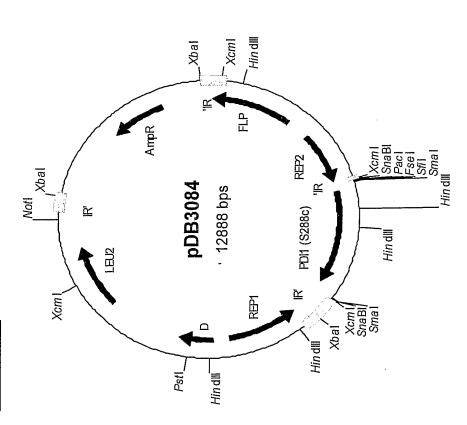


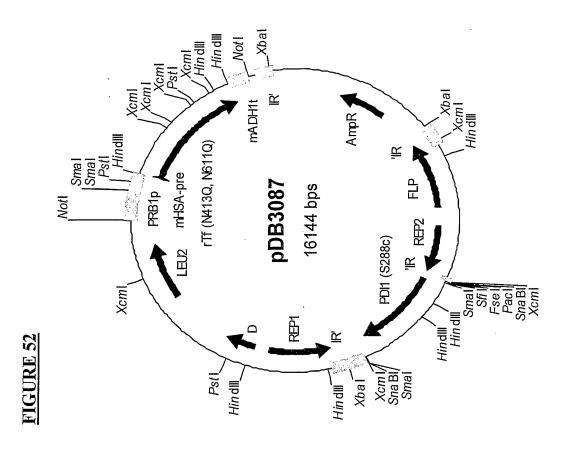




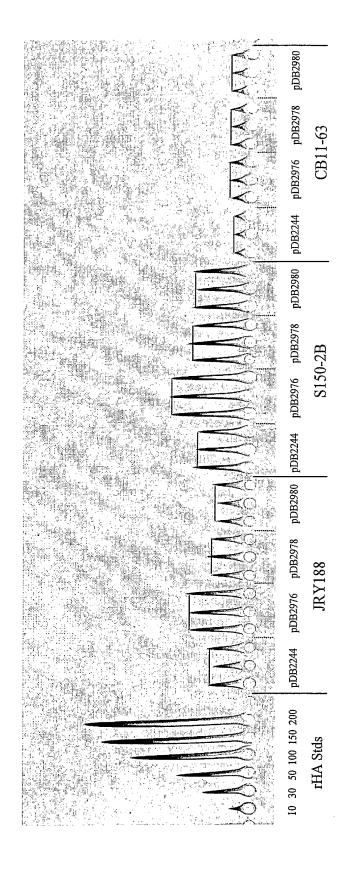




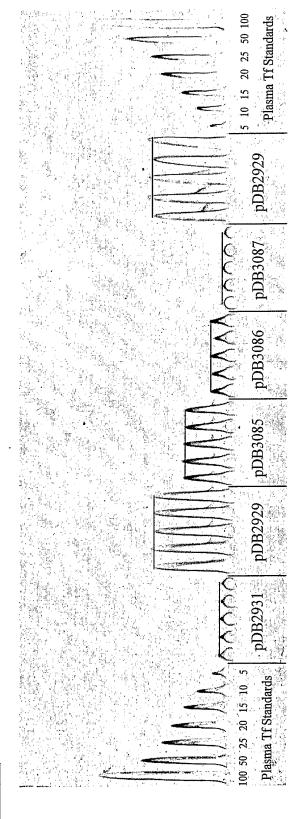




Xcm | Xcm | 区 mADH1t rTf (N413Q, N611Q) 瓦 Smal Smal Pstl mHSA-pre pDB3086 16204 bps PRB1p Notl REP2 PDI1 (S288c) LEU2 Smal Sfill Fsell Pacl SnaBl Xcm HindIII HindIII <u>F</u> <u>ī</u> Xcml/ SnaBl/ Smal Pstl Xbal HindIII Hin dill

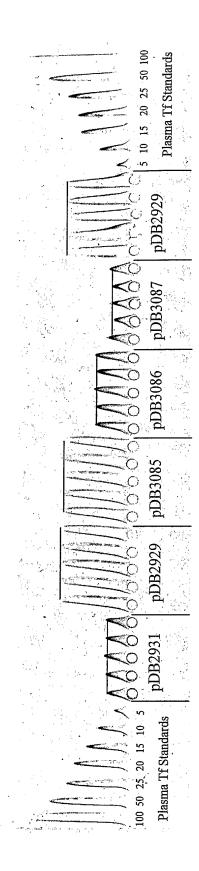


pDB2980



 \mathbf{IGU}

PCT/GB2004/005462



mADHlt Ampr 🦄 rHA Sma I Sma I PstI Fusion Leader Endostatin FLPpDB3100 Notl PRB1p 16559 TR REP2 Nco I LEU2 Eco RV Eco RI Sfil Fsel Pac I Sna BI REP1 Sna Bl Sall Nco I Hin dIII

Eco RI mADH1t IR' rHA AmpR $Eco\,\mathrm{RI}$ Endostatin Fusion Leader LEU2 Hin dIII pDB3099 FLP Noti PRB1p 14578 Eco RV'| Sph I Eco RV Eco RV, Ncol HindIII Pst I Eco RI Hin dIII

R EcoRI mADHIt AmpR A E_{CO} E_{CO} Sma I Sma I Pst I Angiostatin LEU2 Fusion Leader pDB3107 'IR REP2 FLP PRB1p Not I NcoIEco RI Eco RI Sfil Fsel Pac I Sna BI REP1 Eco RV, Sal I Nco I PstI Eco RL HindIII Sna BI HindIII

FIGURE 60

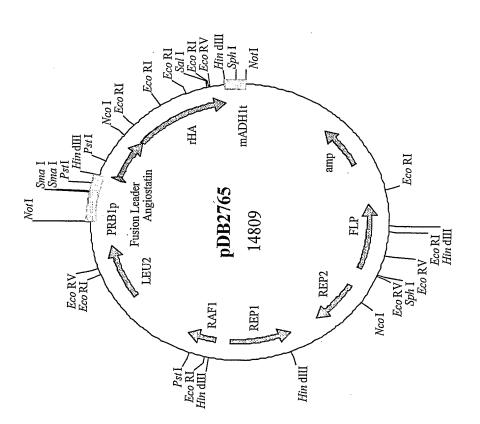


FIGURE 59

EcoRI , Hin dIII Eco RV \mathbb{R} AmpR 🛠 mADH1t Bam HI ,Eco RI Kringle5 GS Linker PRB1p Fusion Leader NotI 16292 TR REP2 Nco I EcoRV EcoRI Sfil Fsel Pac I Sna BI $Eco\,RV / \\ Nco\,I / \\ Hin\,diii / \\ Nco\,I / \\ Hin\,diii / \\ Nco\,I$ REP1 K Ncol Sna Bl Sal I PstI Eco RI, Hin dIII Hin dIII

Eco RI mADH1t 3am HI Hin dIII EcoRIamp **GS** Linker Fusion Leader (** Kringle5 Sma I Sma I Pst I | HindIII Eco RI PRB1p NotI FLP. 14311 EcoRV'| Sph1 EcoRV R REP2 LEU2 EcoRI, Ncol RAF1 REP1 Hin dIII Pst I. Eco RI Hin dIII

FIGURE 61

Eco RI $\langle A_{Hn}$ dIII Eco RI Eco RI Sph I Eco RV AmpR 🥖 Hin dIII ,Bam HII Eco RI mADH1t GS Linker Sma I Sma I PstI Fusion Leader W. DX-890 FLPpDB3102 PRB1p NotI 16220 TR REP2 Ncol Eco RV Eco RI PDII Sfil Fsel Pacl Sna BI $Nco\,I/Min\,dIII/Mco\,I/Min\,dIII/Min\,dIII/Mco\,I/Min\,dIII/Mco\,I/Min\,dIII/Mco\,I/Min\,dIII/Mco\,I/Min\,dIII/Mco\,I/Min\,dIII/Mco\,I/Min\,dII/Min\,dII/Min\,d$ REP1 IR' Eco RV, Sna Bl Sall Nco I Pst I. Eco RI, Hin dIII Hin dIII

FIGURE 64

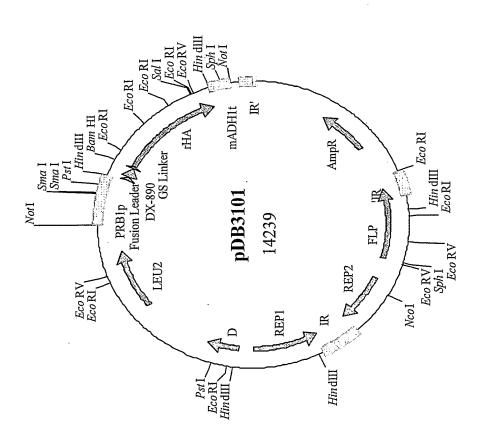
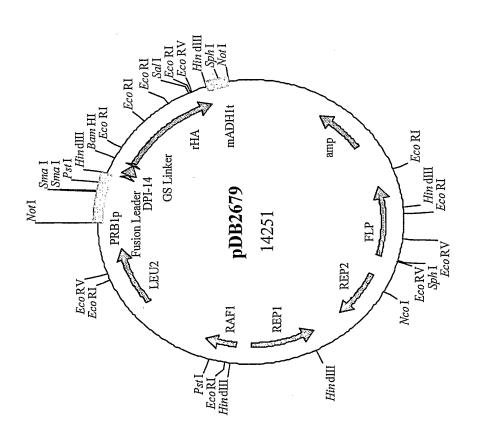


FIGURE 63

 $\begin{bmatrix} Sina & 1 \\ Sina & 1 \\ Sina & 1 \\ Sina & 1 \\ Sina & 1 \end{bmatrix}$ $\begin{bmatrix} Eco & RV \\ Fari \\ Fusion & Leader \\ Fusion & Leader \\ Eco & RV \\ Rep & 1 \end{bmatrix}$ $\begin{bmatrix} Eco & RV \\ Fusion & Leader \\ Fusion & Leader \\ Sin & Rin & Leader \\ Sin & Rin & Leader \\ Rep & Rin & Leader \\ Rep & Rin & Rin & Rep \\ Sin & Bi \\ Rep & Rin & R$

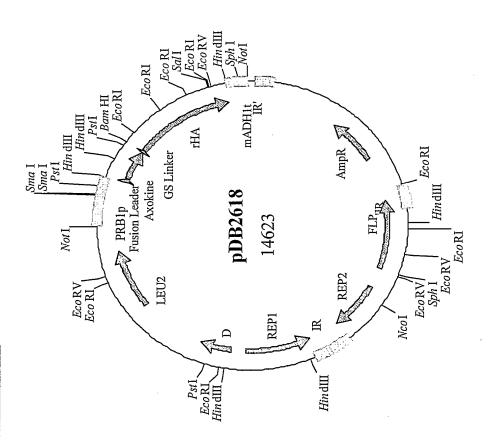
FIGURE 66

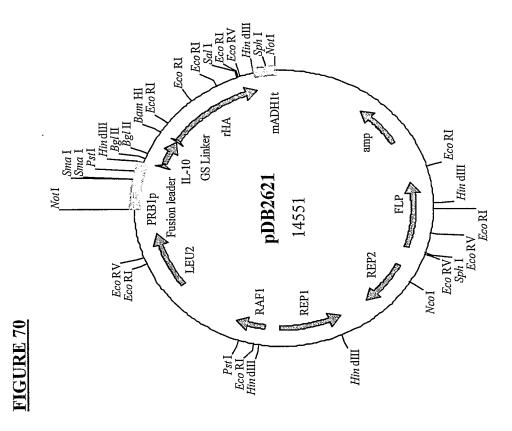


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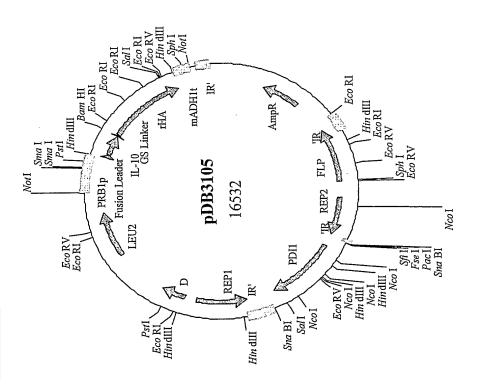
AmpR 🔊 $\begin{cases} & \bigvee_{\substack{Hin \text{ dIII} \\ Eco \text{RV}}} \\ |Sph \text{ I} \\ Eco \text{RV} \end{cases}$ Sma I | Sma I | Sma I | Hin dIII | Hin dIII mADH1t GS Linker 됬 Fusion Leader FLP Axokine pDB3106 PRB1p NotI REP2 NcoI ĸ Eco RI PDII Sfil Fsel Pac I Sna BI REP1 Sna BI Sal I Nco I Pst I, Eco RI Hin dIII Hin dIII

FIGURE 68



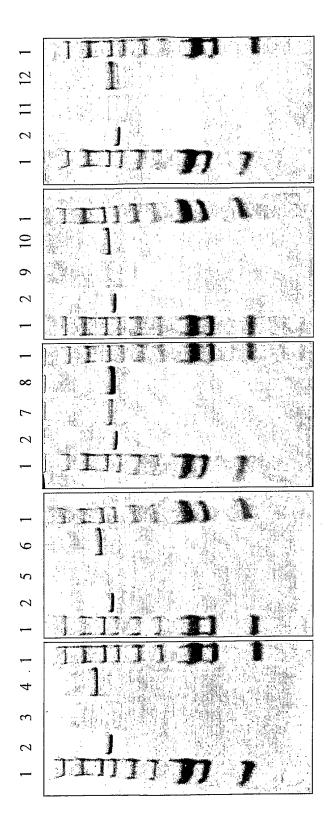


Eco RI Eco RI Eco RI IL-10 GS Linker Smal Smal rHA Fusion Leader Hin dIII PRB1p 6715 bps Smal Notl Sfil NotI SphI mADH1t ori B Amp res

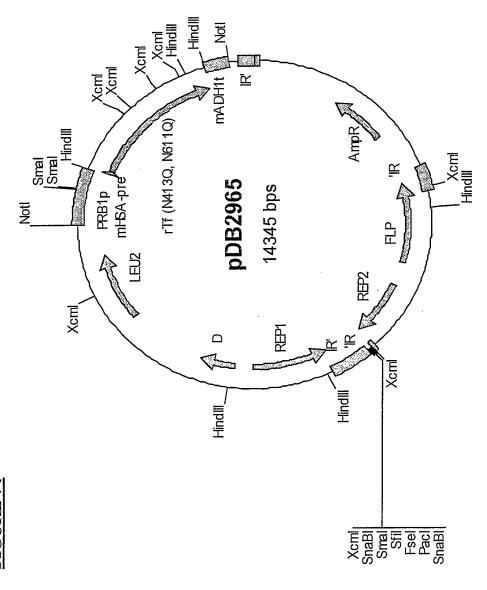


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IGURE 72



TIGURE 72

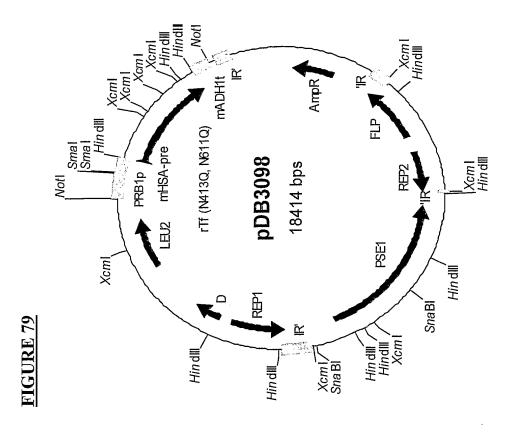


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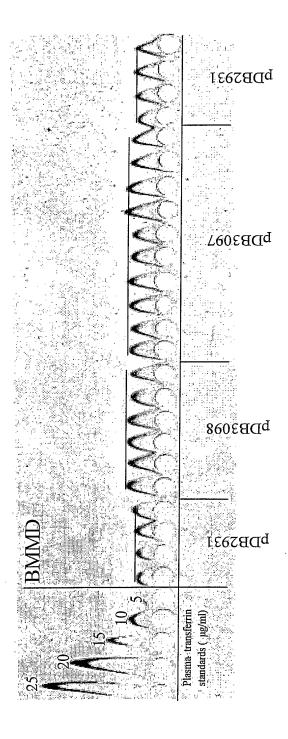
Wdel FcoRi Snall Snall Bam Hi Scal amp amp lacz amp hindill Hindill Hindill Amn Hindill Ndel Xmn Hindill Snall Snall Hindill Mdel Xmn Hindill Hindill Hindill Hindill Hindill Amn Hindill Amn Hindill Amn Hindill Amn Hindill Hindill Hindill Hindill Amn Hindill Amn Hindill Amn Hindill Amn Hindill Amn Hindill Amn Hindill Kmn Hindill Amn Hindill

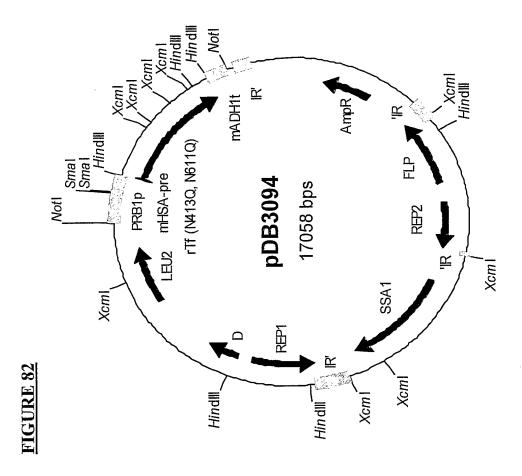
FIGURE 77

Xcml Xcml mADH1t AmpR ; pDB3090-pDB3093 瓦 rTf (N413Q, N611Q) Smal Smal mHSA-pre 15504 bps PRB1p Notl ORMZ SnaBl Pac I SnaBl Xcm I Xcm 西 Xcm1/ Sna Bll Small Sfill Fsel HindIII Hin dIII_



mADH1t IR 톲 FLP Smal Smal Hin dlll rTf (N413Q, N611Q) mHSA-pre PRB1p pDB3097 18414 bps Xcm | Sna Bl RE2 Notl Xcm! / Hin dill Hin dill Xcml Sna Bĺ *Hin* dⅢ Hin dlll Xcm [7 Hin dill Hin dill





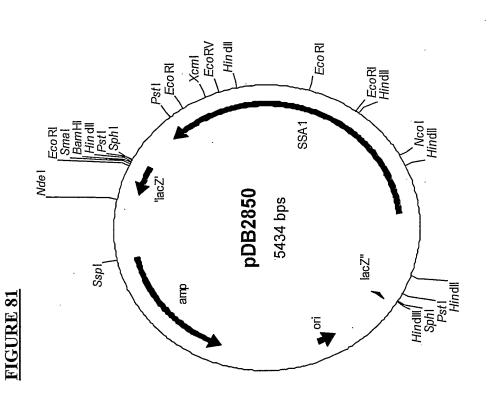
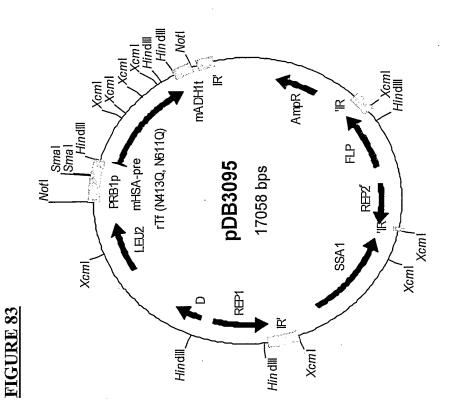
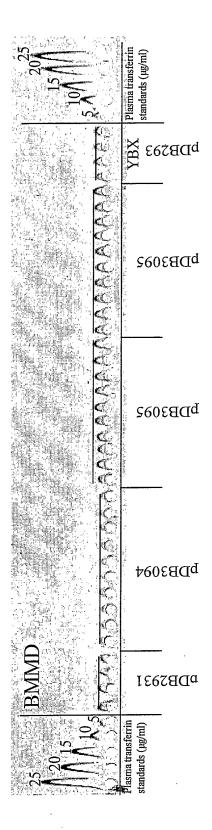


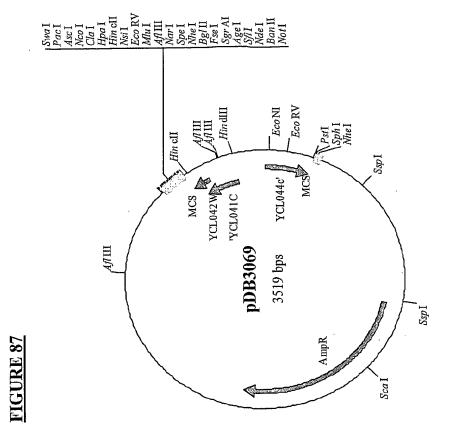
FIGURE 85

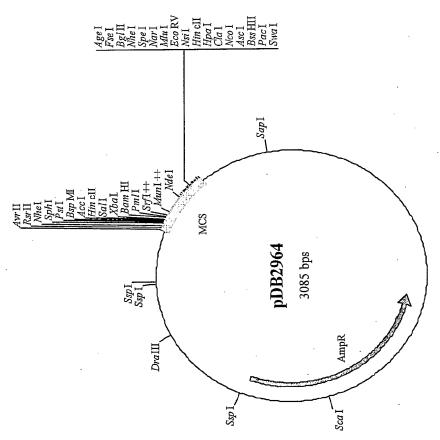


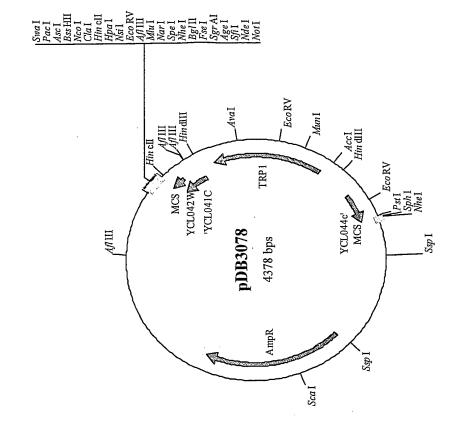
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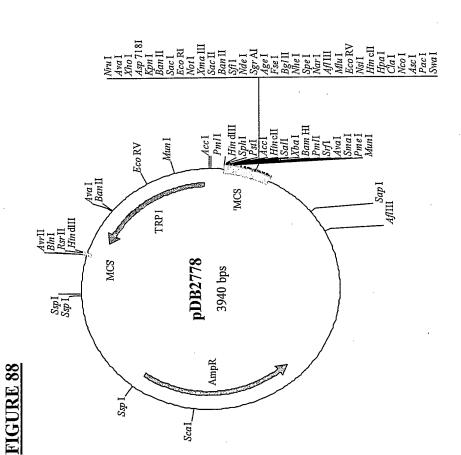


ligure 84



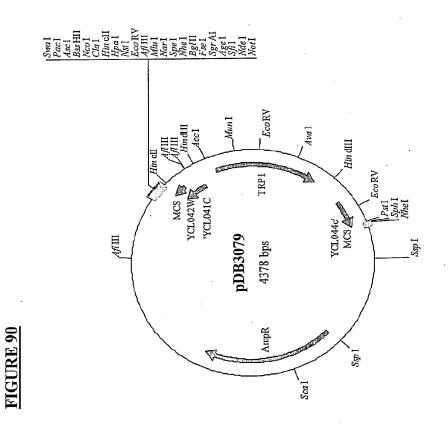






Scal Ampres

FIGURE 91



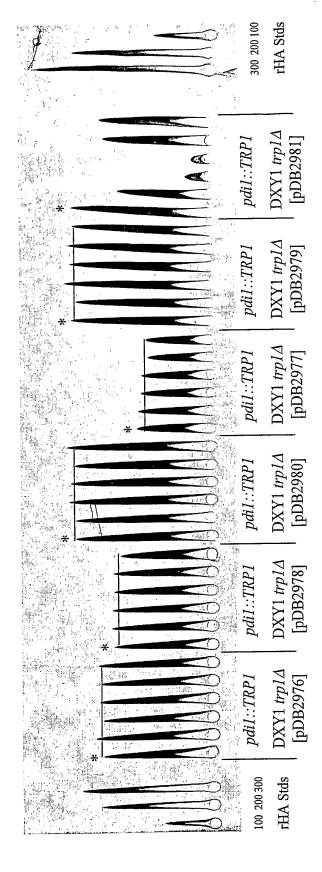


FIGURE 92

Figure 94A Alignment Workspace of Ext.meg.0, Hein (Weighted) 20 December 2004 15:04

					. •
CIRCLICATION CARRENT CONTROLL 20 30 40 50 60 70 80 90 110 110 3 4 5 6 6 70 80 90 110 110 3 4 5 6 6 70 80 90 110 110 4 5 5 6 5 6 70 10 110 110 6 6 7 6 7 6 10 10 110 110 110 10	TATRITTIGITROGOGIGOATITICITGITIGCAAGCAGCAGGTIGICIRATIGGIRANITTIRAAGCIGOCAAGCICIRCATRAAGAAAACATRICAGGTIRITGAAG 120 130 130 140 210 220 220 220 220 220 220 220 220 22	TITICIECIGEIECOGIOCHEICAUGEICCOCHECHOCOCHECHCITITICECCAACAACACEIGIGECOCCHEACACHCOCHGIOCATTAAGTIGECOCAC 230 240 250 240 320 320 330 330 330 330 330 330 330 33	CANCICUTIVAAUGASTRAYITCAGIOGCACCITIGGGGGGGTTTTTTTGCCCCAUGGIGGGCCCCTGTAACAUGGCICGAATRACTGGAATAACCCCCGAATAACAUGACTACCATGAATAACAUGACTACATACATACATACATACATACATACATACATACAT	$\begin{array}{llllllllllllllllllllllllllllllllllll$	AACAGCANIGITIVACAACTICAVICAVITIACAAGGGGACTIVACAAGGCCATIGICCAATTICANGAICAAAGCCAAAGGCCAACGGCCCGTTGTTGCTGANCCT 560 570 580 680 660 660 AACAGCANIGITIVACAAGCTCGAUCGAUTGCGAGGAGCTGAGACTGCCAATTICTCCAATTICANGATCAAGCCAAAGGCCAACGGGTTGTTGCTGANCT 656 AACAGCGANGTTAACAACTGAUCGAUTGCGAGGAGCTGAGAGCCAATTGCCAATTCANGATCAAGCCAAAGCCAACGGGCTTGTTGCTGANCT 660
S288c long	S288c long SKQ2n long	S288c long SKQ2n long	S288c long SKQ2n long	S288c long SKQ2n long	S288c long SKQ2n long

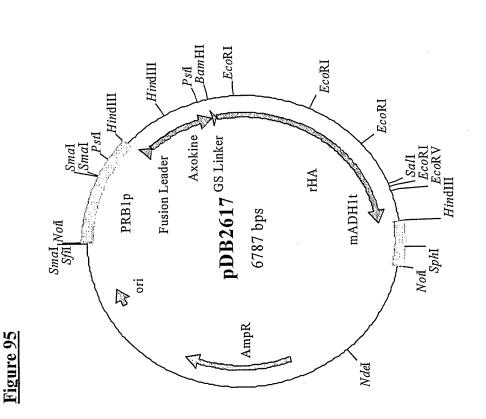
Figure 94B Alignment Workspace of Ext. meg J. Hein (Weighted) 20 December 2004 15:04

Figure 94C

se of Ext. meg u. Hein (Weighted) 15:04	GRICTICARGARCARGATICCICIANTICARTIGATICA 1370 1380 1390 1400 1410 1420 1430 GRICTICARGARACARGATICARGA	SIGSICACIGIRAGAGINIGSCOCCAACTIRACCAAGAACTICATRACCIRACCACACGCOCCACGITITAGNIGCTRAACTICAAAACTACGGAAAACGUGIC 1440 1450 1460 1460 1470 1480 1490 1500 1510 1520 1530 1540 SIGSICACHGIRAGAGARINGSCOCCAACTIRACCAAGACACACACACACACACACACACACACACACACA	reacconcerranteracentrocracatorisme concerrante de la concernation como de la concernation de la concernatio	CATCAAGSAAACGSTCACTICGACGSTCAAGSCCTTGTACGAAGAGACCCCCTGAGAAACCTCCTGAGGAAGCTGACCCCAAGCTGAGCTGATGCTG 1660 1670 1680 1690 1700 1710 1720 1740 1750 1760 CATCAAGSAAACGSTCACTICGACGSTAAGCCTTGTACGAAGACCCCAAAGCTGAGGAAGCTGAGGAAGCTGACGCAAACCTGACGAAGCTGACGCCCAAACCTGACGCCCAAACCTGACGCCCAAACCTGACGCCCCAAACCTGACGCCCCAAACCTGACACCCCAAACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCCCCAAACCTGACACCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCCAAACCTGACACCCAAACCTGACACCCAAACCTGACACCCAAACCTGACACAAACCTGACACCCAAACCTGACACCCAAAACCTGACACAAACCTGACACAAACCTGACACAAACCTGACACAAACCTGACACAAACCTGACACAAACCTGACAAACCTGACAAACCTGACACAAAACCTGACAAACCTGACAAACCTAAAACCAAAACCTAAAAAAAA	AGCIGAATIGACIGAGAAGAIGACATICAGATIGIRALICIGATGACITIGATITICATIRAAIRAGAGAINVIRAGAANITICIRAGAAGITITITITI 1770 1770 1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 ACCIGAATIGACIGAGAAGAGAGAGAATIGAATIGIRATICIGAICACITIGATITICATIRAAIRAGAAIRIRAGAAATITICIRAGAAGATITITI 184: ACGCIGAATIGACIGAGAAGAAGAGAATIGAAATIGIRATICIGAICACITITIGATITITICATIRAAIRAGAAIRIRAGAAATITITICIRAGAAATITITITI 1870	AARAAAAAICAIRAAAAAAAAAAAAAAAAAAAAAAAAAA
Alignment Workspace of Ext.meg U. H 20 December 2004 15:04	GANCINGARAACCAABA GANCINGARAACCAAGG SKQ2n long GANCINGARAACCAAGG	GIGGICACIGIRAGAGA 1440 1 S288c long GIGGICACIGIRAGAGA SKQ2n long GIGGICACIGIRAGAGA	ACAGGGGGATGATTGAA 1550 S288c long ACAGGGGGGAATTGAA SKQ2n long ACAGGGGTGAATTGAA	CAUCAAGSAAACGSIC 1660 S288c long CAUCAAGSAAACGSIC SKQ2n long CAUCAAGSAAACGSIC	AGCIGANTIGACIGNO 1770 S288c long ACCCIGANTIGACIGNO SKQ2n long ACCCIGANTIGACIGNO	ASARC TOTAL ABARARA TOTAL SERVICE SERV

EcoRI IR' mADHlt AmpR 🚿 HindIII rHA EcoRI GS Linker Sma I Sma I Pst I Fusion Leader Axokine HindIII pDB2618 14623 bps PRB1p FLP NotI, LEU2 R REP2 EcoRV| REP1 Ncol 黑 HindIII

Figure 96



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